

ATCC[®] connection[™]

ATCC and amaxa Partner To Offer Transfection Protocols

ATCC and amaxa GmbH have entered into an agreement whereby ATCC will supply amaxa with cell lines to develop and optimize protocols using amaxa's Nucleofector[®] technology. The primary goal of the collaboration is to enable researchers to work with optimized and ready-to-use nonviral transfection protocols on a wide range of cell lines.

Transfection is the transfer of foreign nucleic acids into eukaryotic cells. During the process, DNA, mRNA or siRNA must pass through the cell membrane into the cytoplasm or organelles, and in general either chemical or physical techniques are used to accomplish this transfer. Transfection allows for the expression of exogenous proteins (or, in the case of siRNA, the suppression of expression) by eukaryotic cells

and thus is a valuable tool in the study of protein function and genomics. Nucleofection delivers nucleic acids directly into the nucleus as well as into the cytoplasm, ensuring that expression starts shortly after transfection even in nondividing cells.

"By cross-referencing optimized protocols developed by amaxa with detailed information about ATCC cell lines, we are making it easier for researchers to be successful with genuine ATCC cultures and amaxa Nucleofector technology," said Mike Gove, Vice President, Sales and Marketing, ATCC.

For most research projects, ATCC recommends starting with new, low-passage

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Effects of Reduced Oxygen Tension on HUES-7 Human Embryonic Stem Cells

C.J. Ordning, H.K. Josephson, and J.M. Auerbach, ATCC

Human embryonic stem cells (hESC) are often cultured with feeder cells in oxygen levels that are higher than those in living tissues. The traditional gas mix consisting of 5 to 10% CO₂ in air contains 18 to 21% oxygen. The concentration of oxygen in the human fallopian tube is equivalent to 5% (1), and a variety of stem cells exist in vivo in 2 to 6% oxygen.

In studies of in vitro embryo growth, reduced oxygen tension does not affect blastocyst development but does significantly increase the number of cells in the inner cell mass, from which hESC are derived (1,2). The relative and absolute growth of hESC, as determined by mean percentage of undifferentiated growth from the original colony and by actual physical measurement, is improved by culture in a

reduced oxygen environment (3), and low oxygen prevents differentiation of hESC colonies and enhances formation of embryoid bodies (4). Excess O₂ can generate reactive oxygen species leading to cellular damage (5), increase the rate of telomere shortening (6), generate chromosomal instability, produce chromatid and chromosome gaps and breaks (7,8) and inhibit proliferation in G1, S, and G2 phases of the cell cycle (6,9,10).

Feeder cells lend support to hESC for proliferation in the pluripotent, undifferentiated state but can also be a source of a contaminating cell population. Therefore it is of great advantage to culture hESC independent of feeder cells derived

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What's new

DNA Clone Collections

Mouse transcription factor clones from the Dana-Farber Cancer Institute and GENSAT-BAC clones from Rockefeller University and NINDS are now searchable online. From the home page at www.atcc.org, choose Molecular Genomics from the dropdown menu at the upper right. You can search by identifier or by keyword.

Tumor Cell Lines

These lung adenocarcinoma cell lines were developed in the laboratory of A. Gazdar and J. Minna. Each has an acquired mutation in the EGFR tyrosine kinase domain.

| ATCC® No. | Name | Price |
|-----------|---------|-------|
| CRL-2868™ | HCC827 | \$235 |
| CRL-2869™ | HCC2935 | \$235 |
| CRL-2871™ | HCC4006 | \$235 |

Plasmodium vivax

Six strains of *Plasmodium vivax* that are cultured in primates have been deposited by NIH and are now available from ATCC.

| ATCC® No. | Strain | Price |
|-----------|------------------|-------|
| 30060™ | Chesson | \$150 |
| 30073™ | NICA | \$150 |
| 30151™ | South Vietnam | \$150 |
| 30139™ | South Vietnam II | \$150 |
| 30152™ | Sal 1 | \$150 |
| 30197™ | SAL II | \$150 |

Oregon Collection of Methanogens

The Oregon Collection of Methanogens (OCM) is in the process of being transferred to ATCC. The OCM is the premier collection of methanogenic archaea in the world, containing novel methanogens curated by the late Dr. David Boone of Portland State University.

Key features of the OCM:

- 270 strains of methanogens, including most of the type strains
- 17 new type strains of other prokaryotes, including halophiles and sulfate-reducing bacteria
- A large collection of unidentified anaerobes from the deep subsurface

A grant from the Living Stock Collections program at the National Science Foundation supports this endeavor. We are grateful to Portland State University and to Dr. Boone for his foresight in entrusting this valuable collection to ATCC's care, and for ensuring that these fascinating organisms remain available to scientists.

Currently 19 OCM strains are available from ATCC:

| ATCC® No. | Organism | Strain | Price |
|-----------|---|---------|-------|
| 43576™ | <i>Methanocorpusculum labreanum</i> | Z | \$198 |
| 43379™ | <i>Methanobacterium alkaliphilum</i> | WeN4 | \$198 |
| 43573™ | <i>Methanosarcina mazei</i> | LYC | \$198 |
| BAA-911™ | <i>Methanobolus taylorii</i> | GS-16 | \$160 |
| BAA-912™ | <i>Methanohalophilus portucalensis</i> | FDF-1 | \$160 |
| BAA-914™ | <i>Methanogenium organophilum</i> | CV | \$160 |
| BAA-927™ | <i>Methanothermobacter marburgensis</i> | Marburg | \$160 |
| BAA-928™ | <i>Methanobolus oregonensis</i> | WAL1 | \$160 |
| BAA-929™ | <i>Methanopusculum bavaricum</i> | SZSXXZ | \$160 |
| BAA-930™ | <i>Methanobacterium ivanovii</i> | Ivanov | \$160 |
| BAA-931™ | <i>Methanosarcina siciliae</i> | T4/M | \$160 |
| BAA-932™ | <i>Methanobolus vulcani</i> | PL-12/M | \$160 |
| BAA-933™ | <i>Methanocorpusculum sinense</i> | China Z | \$160 |
| BAA-1071™ | <i>Methanohalophilus halophilus</i> | Z-7982 | \$160 |
| BAA-1072™ | <i>Methanohalobium evestigatum</i> | Z-7303 | \$160 |
| BAA-1073™ | <i>Methanobacterium espanolae</i> | GP9 | \$160 |
| BAA-1076™ | <i>Methanothermobacter thermophilus</i> | M | \$160 |
| BAA-1077™ | <i>Methanobacterium palustre</i> | F | \$160 |
| BAA-1078™ | <i>Methanofollis tationis</i> | | \$160 |

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Update on Toxigenic Bacteria

The Centers for Disease Control and Prevention (CDC) has provided ATCC with clarification on the regulations regarding the distribution of toxigenic strains of non-select agent bacteria. As a result of this clarification, certain strains of *Clostridium perfringens*, *Escherichia coli*, and *Staphylococcus aureus* have been released for general distribution.

The U.S. Department of Commerce regulates the distribution of some bacteria, and many toxigenic strains are available only within the United States. A Customer Acceptance of Responsibility form must be completed and returned to ATCC before these items can be shipped. For a copy of this form contact the sales department at 800-638-6597 or sales@atcc.org.

Regulatory information on every ATCC microorganism can be found in the online catalog. Use the search to find the item of interest. From the product description, click on the link in the Forms/Permits field to find the requirements for your country.

| <i>Clostridium perfringens</i> | | <i>Escherichia coli</i> | |
|--|-----------|-------------------------|-----------|
| Strain Designation | ATCC® No. | Strain Designation | ATCC® No. |
| strain 34 | 3626™ | B2F1 | 51435™ |
| 665 | 17865™ | B99BE001161 | 700840™ |
| 801 | 17870™ | EDL 931 | 35150™ |
| NCTC 8237 | 13124™ | CDC 1994-3023 | BAA-182™ |
| NCTC 8237 (DNA) | 13124D | CDC 1994-3024 | BAA-178™ |
| NCTC 8239 | 12917™ | CDC 1997-3215 | BAA-179™ |
| NCTC 8678 | 12919™ | CDC 1999-3302 | BAA-180™ |
| NCTC 8679 | 12920™ | CDC 1999-3249 | BAA-181™ |
| NCTC 10239 | 14809™ | CDC 1997-3148 | BAA-183™ |
| VPI 2641 | 25768™ | CDC 2000-3025 | BAA-184™ |
| | | CDC 2001-3004 | BAA-176™ |
| | | CDC 2000-3159 | BAA-177™ |
| | | CDC 92-3265 | 700376™ |
| | | CDC 92-3099 | 700377™ |
| | | CDC 92-3073 | 700378™ |
| | | CDC B1409-C1 | 43889™ |
| | | CDC C984 | 43890™ |
| | | CDC EDL 932 | 43894™ |
| | | CDC EDL 933 | 43895™ |
| | | EDL 933 | 700927™ |
| | | EDL 933 (DNA) | 700927D |
| | | H414-36/89 | 51434™ |
| | | RIMD 0509952 | BAA-460™ |
| | | RIMD 0509952 (DNA) | BAA-460D |
| <i>Staphylococcus aureus subsp. aureus</i> | | | |
| 137 | 19095™ | | |
| 7294 | 51650™ | | |
| C.P | 8095™ | | |
| FDA 196E | 13565™ | | |
| FDA 243 | 14458™ | | |
| FDA 587 | 27154™ | | |
| FRI-326 | 27664™ | | |
| FRI-569 | 51811™ | | |
| M.B | 8096™ | | |
| RN 3984 | 51651™ | | |
| <i>Staphylococcus sp.</i> | | | |
| 494 | 23235™ | | |
| FDA 235A | 13567™ | | |
| FDA S6 | 13566™ | | |

ATCC and Competitive Technologies Offer Escrow Services for Biological Materials

ATCC and Competitive Technologies, Inc., have launched a new escrow service for biological materials that are the subject of license agreements. This relationship will combine ATCC's expertise in managing the storage and distribution of biological materials with CTT's technology commercialization expertise.

The escrow service is designed to assist technology companies and universities in protecting valuable biological materials, as well as associated information. The escrow service will assure organizations engaged in licensing activities that their biological materials will remain secure and available to the parties as necessary during the term of a license agreement.

"License agreements involving biological materials require a mechanism for assuring the security and continued availability of these materials during the effective term of the agreement," said Dr. Jesús Soriano, ATCC's Vice President of Licensing, Contracts and Compliance. "CTT's expertise in commercializing intellectual property assures that the escrow service fully supports all elements of a licensing agreement."

"ATCC's extensive experience in cryopreservation and storage of biological materials provides the necessary assurance that deposits of these materials for escrow purposes are maintained under the highest standards of security, safety and confidentiality," according to Aris Despo, CTT's Senior VP for Life Sciences. "We are excited to bring this new escrow service to CTT's expanding relationships with industry and universities."

Competitive Technologies (www.competitivetech.net) is a global leader in identifying, developing and commercializing innovative technologies in life, electronic, nano, and physical sciences. To inquire about escrow service call 203-255-7806.

Stem Cells

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from another individual or species.

In this study, we compared the effects of oxygen tension on plating efficiency and pluripotency of hESC line HUES-7 cultured without feeder cells.

Materials and Methods

The HUES-7 cell line was cultured in 5% CO₂ in air and in 5% CO₂/3% O₂/92% N₂ for several passages. Plating efficiency was determined at each passage. Characterization by immunocytochemistry for human pluripotent ESC-specific markers was performed.

Culture of hESC. HUES-7 cells were cultured without feeder cells in tissue culture vessels coated with 20 µg/ml of human fibronectin (hFN, R&D Systems). The culture medium was changed daily and supplemented with 100 ng/ml basic-FGF (bFGF, R&D Systems). Cells were split using 0.05% trypsin-EDTA. The first two passages were plated at high density; for the third passage, cells were plated at low density (1.0 x 10⁵ cells) in a 10-cm tissue culture dish.

Immunocytochemistry in situ. Cultures were fixed in 4% paraformaldehyde in PBS (ATCC, catalog no. SCRR-2201) for 20 minutes. For intracellular staining, cells were permeabilized using 0.5% saponin. Fixed preps were then stained with antibodies against the human pluripotent ESC markers Oct4 (BD Transduction Laboratories) and SSEA-4 (R&D Systems) as well as the human specific marker TRA-1-85* (Developmental Studies Hybridoma Bank) and an Alexa Fluor 488 goat anti-mouse secondary antibody (Molecular Probes).

Flow cytometry. Cultures were dissociated to single cells using 0.05% trypsin-EDTA, then fixed in 2% paraformaldehyde for 20 minutes. For intracellular staining, cells were permeabilized using 0.5% saponin. Fixed cells were stained with antibodies against the human pluripotent ESC markers Oct4, TRA-1-60 (Chemicon), TRA-1-81 (Chemicon), and SSEA-4 (PE-conjugated, R&D Systems) as well as the human specific marker TRA-1-85 and an Alexa Fluor 488 goat anti-mouse secondary antibody.

Karyotyping. Karyotype analysis was performed using colcemid arrest and G-banding.

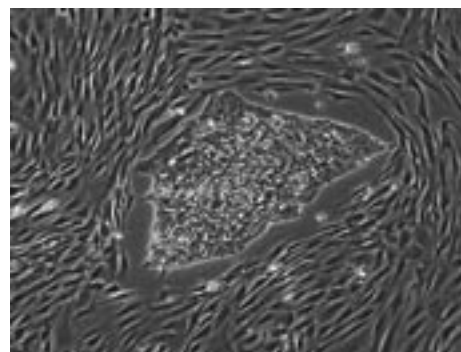
Results

When plated at low density without feeder cells on hFN-coated 10-cm culture dishes, HUES-7 hESC failed to grow in an environment of 5% CO₂ in air.

The cultures resulting from the same plating conditions in 5% CO₂/3% O₂ were heterogeneous, containing proliferating pluripotent HUES-7 hESC cells in colonies and an unidentified, human, nonpluripotent cell type that proliferates in a monolayer until confluency (Figure 1). After six passages in these conditions, the colonies stained positive for stem cell markers Oct4 and SSEA-4 while the monolayer cells did not. All cells in these heterogeneous cultures stained positive for anti-human TRA-1-85 (Figure 2). The monolayer cells did not inhibit or restrict the proliferation of the hESC colonies, which continued to proliferate in the undifferentiated state as determined by immunophenotyping (Figure 3).

After four passages on hFN, the karyotype of HUES-7 was comparable to the karyotype of cells initially cultured on mouse embryonic fibroblasts.

Figure 1. Phase images of HUES-7 on hFN. Undifferentiated hESC colonies proliferated among a monolayer of nonpluripotent hESC-derived cells.

**Conclusion**

The 5% CO₂ in air environment did not support the growth of HUES-7 plated at low density without feeder cells. However, a reduced oxygen culture environment of 5% CO₂/3% O₂ supported survival and proliferation of hESC on hFN at low plating density without feeder cells. The resulting culture was a mix of undifferentiated hESC and an unidentified human cell type which proliferates until confluent in culture.

In these mixed cultures, HUES-7 hESC continue to proliferate in the undifferentiated state in a manner similar to HUES-7 co-

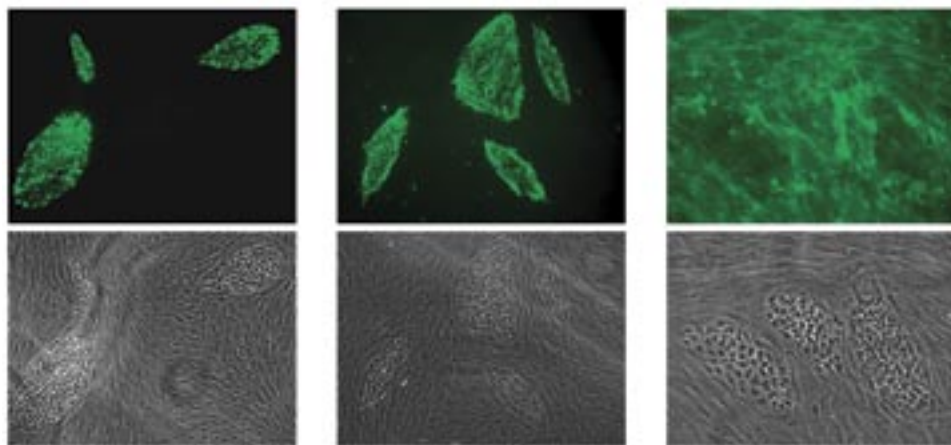
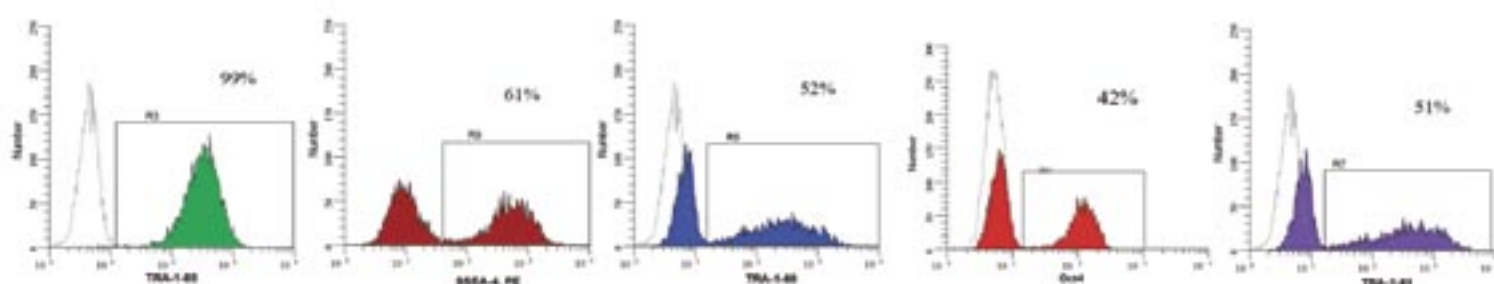


Figure 2. In situ immunostaining of HUES-7 (hFN). A. hESC colonies stain positive for Oct4 while monolayer is negative. B. hESC colonies stain positive for SSEA-4 while monolayer is negative. C. Both the hESC colonies and monolayer stain positive for anti-human TRA-1-85.

Figure 3. Flow cytometry analysis of HUES-7on hFN for human marker TRA-1-85 and for human pluripotent stem cell markers SSEA-4, TRA-1-60, Oct4, and TRA-1-81.



cultured with mitotically arrested feeders. Thus a culture environment of reduced oxygen tension, which more closely resembles the physiological state, enhances the plating efficiency and proliferation of pluripotent hESC cultured without feeder cells as compared to normoxic conditions.

Further studies will include characterizing the non-hESC cell type and culturing the non-hESC and the hESC independently to determine if their viability remains at the same level and if the hESC maintain pluripotency. Also, the experimental conditions may be extended to test additional markers through more passages.

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* The TRA-1-85 antibody (developed by Peter W. Andrews) was obtained from the Developmental Studies Hybridoma Bank, which was established under the auspices of NICHD and is maintained by the University of Iowa Department of Biological Sciences.

This project was presented as a poster at the 2005 meeting of the International Society for Stem Cell Research and is partially supported by the National Institute on Aging (N01AG40002).

ATCC® Bringing confidence to stem cell research

The ATCC Stem Cell Center, funded in part by NIH, provides a comprehensive array of products and services:

- Affordable and well-characterized **human and mouse stem cell lines** screened for appropriate markers and maintained in an undifferentiated state
- Qualified mouse and human **feeder layer cells**
- High-quality **media, serum, and reagents** tested for stem cell use
- Expert **technical support and protocols** from experienced scientists



Visit stemcells.atcc.org for more information.

Tech Qs

How can I get my HK-2 cells to proliferate?

ATCC cultures HK-2 cells (ATCC®CRL-2190™) in Keratinocyte Serum-Free Medium (GIBCO 17005-042) supplemented with 5 ng/ml recombinant epidermal growth factor (EGF) and 0.05 mg/ml bovine pituitary extract. HK-2 cells express epidermal growth factor receptors; the addition of EGF to the culture medium signals proliferation. Because EGF will not pass through a 0.2- μ m filter, add it aseptically to sterile medium.

Can I use the human embryonic stem cell line hESC BG01V if my research is funded by the U.S. government?

Yes, the hESC BG01V cell line is eligible for use in federally funded research. It is available from the ATCC Stem Cell Center as ATCC® SCRC-2002™. For additional information pertaining to derivations of stem cells that qualify for federal funding, visit the NIH Human Embryonic Stem Cell Registry at stemcells.nih.gov/research/registry/.

I received an adherent cell line from ATCC, but the cells do not seem to be attaching. What should I do?

Most adherent cell lines from ATCC will attach after 24 to 48 hours of incubation. However, some cells may remain in suspension or display a mixed morphology of adherent and suspension cells for an extended period of time in recovery. ATCC recommends that you allow cells recovering from cryopreservation to remain in culture for at least four days before discarding them as nonviable. It's a good idea to test the viability of cells in suspension using an exclusion dye such as Trypan Blue (catalog no. 30-2402) or Erythrosin B (catalog no. 30-2404) before discarding. You may also ask an ATCC Technical Services representative about the morphology of cells recovering from cryopreservation.

If viable cells are still in suspension after 72 hours or more and you are certain that the cells should be attached, you may need to check your culture conditions. Slight changes in pH, osmolality, temperature, and availability

of nutrients can have profound effects on cells in culture. Use the following guidelines to assess the culture conditions of your cells.

Complete culture medium recommended on the product sheet. Keep in mind that there are many different versions of the same commercial medium. As a result, your medium may lack critical components (such as L-glutamine) or contain different concentrations of key components (such as sodium bicarbonate). Recheck your medium formulation or contact ATCC Technical Services for assistance. Ordering ATCC medium and serum will provide your cells with the same optimal conditions they received in our labs.

If you are not using the medium that ATCC recommends, it's possible that the cells are going through a period of adaptation and may need to be treated as a suspension or semi-adherent cell line until attachment occurs. Alternatively, the cells could be displaying a variant phenotype when grown in altered concentrations of growth factors, attachment factors, amino acids, and/or vitamins. Research may be necessary to see if other researchers have observed similar phenomena.

Dilution of the frozen cell suspension. Batch-specific information provided with the product sheet contains specific dilution recommendations. Adding too much or too little medium during recovery may significantly delay attachment and hinder subsequent outgrowth of cells during recovery.

Antibiotics and antimycotics. Although antimicrobics are typically used to target prokaryotes, some antibiotics (e.g., G418 or Hygromycin B) and antimycotics (Amphotericin B or Fungizone) can potentially have a toxic effect on eukaryotic cells in culture. If your lab routinely utilizes antibiotics for cell culture, you may want to limit use to 1% Penicillin-Streptomycin Solution (catalog no. 30-2300).

Incubator conditions. The temperature and carbon dioxide levels in the incubator are important for optimal conditions of growth. Most animal cell lines require a temperature of 37°C for incubation. There are a few cell lines at ATCC, especially insect

and amphibian cell lines, which require lower temperatures. Temperatures above or below the recommended temperature can produce detrimental results.

Carbon dioxide requirements will vary depending on the culture formulation; follow the specifications on the product sheet. For more detailed information pertaining to carbon dioxide levels, visit the cell biology FAQs in the technical support section of our Web site at www.atcc.org.

Culture vessel. ATCC typically recovers cells in tissue-culture-grade flasks. If you are using dishes instead of flasks, make sure that the vessels are appropriate for use with cell cultures. Bacterial-grade dishes, for example, are not appropriately charged to induce attachment of eukaryotic cells. Likewise, make sure you are using a fresh package of dishes or flasks; opened packages may contain vessels whose net negative surface charge has been compromised or reduced.

TechQs are intended for informational purposes only. ATCC is not responsible for results obtained from the use of this information.

Meetings and Conferences

ATCC will be attending the following meetings in early 2006. Stop by and talk to an ATCC representative about how we can help your research.

Food Safety Summit and Expo
March 22 – 24, Las Vegas, NV

Experimental Biology 2006
April 1 – 5, San Francisco, CA

American Association for Cancer Research
April 1 – 5, Washington, DC

International Society for Biological and Environmental Repositories
April 30 – May 3, Bethesda, MD

American Society for Microbiology
May 21 – 25, Orlando, FL

Transfection

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cultures to minimize the risk of genetic or phenotypic changes. The Nucleofector technology and other transfection protocols should be performed using fresh and authenticated cells.

Optimized protocols for the following ATCC cell lines are now available. See the amaxa Web site at www.amaxa.com for details.

| | | | |
|-----------------|------------|------------|-------------|
| 32D | CRL-11346™ | MCF-7 | HTB-22™ |
| 3T3-L1 (pre-ad) | CL-173™ | MDCK | CCL-34™ |
| A-10 | CRL-1476™ | MDA-MB-231 | HTB-26™ |
| A-431 | CRL-1555™ | MDA-MB-453 | HTB-131™ |
| A20 | TIB-208™ | MDA-MB-468 | HTB-132™ |
| A549 | CCL-185™ | MOLT-4 | CRL-1582™ |
| AGS | CRL-1739™ | NCI-H1299 | CRL-5803™ |
| BHK-21 | CCL-10™ | Neuro-2a | CCL-131™ |
| BJ | CRL-2522™ | NIH/3T3 | CRL-1658™ |
| C2C12 | CRL-1772™ | NRK | CRL-6509™ |
| C6 | CCL-107™ | P19 | CRL-1825™ |
| Caco-2 | HTB-37™ | PANC-1 | CRL-1469™ |
| CCRF-CEM | CCL-119™ | PC-12 | CRL-1721™ |
| CHO | CCL-61™ | PC-3 | CRL-1435™ |
| COS-1 | CRL-1650™ | Raji | CCL-86™ |
| DU 145 | HTB-81™ | Ramos | CRL-1596™ |
| EL4 | TIB-39™ | RAW 264.7 | TIB-71™ |
| FDC-P1 | CRL-12103™ | RBL-1 | CRL-1378™ |
| HCT-116 | CCL-247™ | Saos-2 | HTB-85™ |
| HEK293 | CRL-1573™ | SH-SY5Y | CRL-2266™ |
| HeLa | CCL-2™ | SK-N-SH | HTB-11™ |
| HepG2 | HB-8065™ | SK-OV-3 | HTB-77™ |
| HL-60 | CCL-240™ | SW480 | CCL-228™ |
| HT-1080 | CCL-121™ | T-47D | HTB-133™ |
| HT-29 | HTB-38™ | THP-1 | TIB-202™ |
| HuT 78 | TIB-161™ | U-2 OS | HTB-96™ |
| IMR-90 | CCL-186™ | U-937 | CRL-1593.2™ |
| Jurkat | TIB-152™ | U266B1 | TIB-196™ |
| K-562 | CCL-243™ | U87-MG | HTB-14™ |
| KG-1 | CCL-246™ | Vero | CCL-81™ |
| LNCaP | CRL-1740™ | WEHI-231 | CRL-1702™ |

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Save on the following products:

| Description | Catalog No. |
|---|--------------|
| DMEM: F12 Medium | 30-2006 |
| Dulbecco's Modified Eagle's Medium | 30-2002 |
| Eagle's Minimum Essential Medium | 30-2003 |
| ES-DMEM without L-glutamine, with 4500 mg/l glucose | SCRR-2010 |
| F-12K Medium | 30-2004 |
| Iscove's Modified Dulbecco's Medium | 30-2005 |
| Leibovitz's L-15 Medium | 30-2008 |
| McCoy's 5A Medium, Modified | 30-2007 |
| RPMI-1640 Medium | 30-2001 |
| Fetal Bovine Serum, 500 ml | 30-2020 |
| Fetal Bovine Serum, 100 ml | 30-2021 |
| Fetal Bovine Serum, ES Qualified | SCRR-30-2020 |

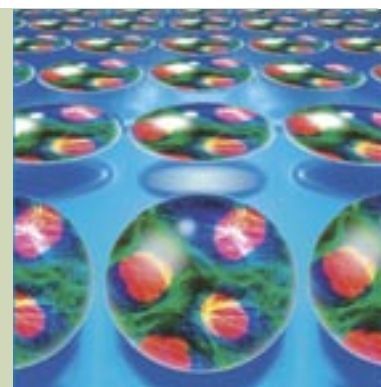
And more! For the complete product line visit the Cell Biology section of the ATCC Web site at www.atcc.org.

Another TERT-Immortalized Cell Line Now Available

ATCC has added hTERT-HME1 (ME16C), human mammary epithelium ATCC® CRL-4010™ to the list of ATCC cell immortalization products. ATCC now offers three hTERT-immortalized cell lines and a eukaryotic expression plasmid containing the hTERT cDNA so that researchers can immortalize their own cell lines:

- hTERT RPE-1, human retinal pigmented epithelium (ATCC® CRL-4000™)
- BJ-5ta, human foreskin fibroblast (ATCC® CRL-4001™)
- pGRN145, plasmid in *E. coli* GC10 (ATCC® MBA-141)

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Yeast Deletions with the SGA Reporter Construct

ATCC has added a new collection of yeast ORF deletion strains. Derived from strains generated by the *Saccharomyces* Genome Deletion Project, these *Saccharomyces cerevisiae* heterozygous diploid ORF deletions with the SGA reporter construct were developed by Boeke and colleagues (1) via introduction of a 4,600-bp fragment of *can1L::LEU2-MFA1pr-HIS3::can1R* into heterozygous diploid ORF deletion strains.

This set of sixty-five 96-well plates (catalog number GSA-8) is a unique tool for functional analysis of the yeast genome. The inclusion of the SGA reporter construct allows for the selection of haploid MATa cells in a mixed population of parental cells and their sporulation progenies. This selection facilitates a wide range of research applications: dosage synthetic lethality, genetic suppression of lethality or growth weakness, synthetic haplo-insufficiency, chemical genomic screening, physiological stress screening, and other applications (1,2).

Strain Development

These strains were derived from direct transformation of the KanMX yeast ORF knock-out collection of heterozygous diploid *S. cerevisiae* with the SGA reporter, or 'magic' marker (Figure 1). The parental strain is BY4743, a direct descendant of S288C (3). The overall

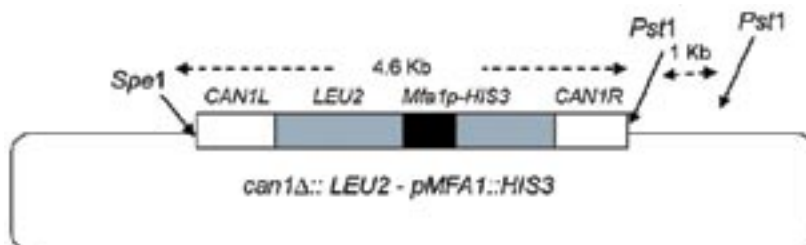


Figure 1. The SGA reporter

genotype of the collection is *MATa/ura3Δ0/ura3Δ0 leu2Δ0/leu2Δ0 his3Δ1/his3Δ1 lys2Δ0/LYS2 met15Δ0/MET15 can1Δ::LEU2-MFA1pr-HIS3/CAN1 xxx::KanMX/XXX*.

Molecular barcodes tag each strain to facilitate analysis by individual gene or entire genome. Primer sites allow easy amplification of the inserts.

Quality Testing

ATCC's authentication process includes test methods such as PCR and DNA sequencing of the molecular barcodes. Strains are tested by both random and directional sampling of wells on each plate to ensure plate identity and orientation during the replication process, as well as strain identity. The strains have also been tested for appropriate nutrient requirements in both the depositor's laboratory and in ATCC laboratories.

More Yeast Genetic Resources

The new ORF deletion strains with the SGA reporter join an extensive collection of deletion strains available from ATCC. See the Yeast Genetic Stock Center page at www.atcc.org for a summary of all available sets.

References

1. Pan X et al. A robust toolkit for functional profiling of the yeast genome. *Mol. Cell* 16(3): 487-496, 2004 PubMed 15525520.
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3. Brachmann CB et al. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains for PCR-mediated gene disruption and other applications. *Yeast* 14(2): 115-132, 1998, PubMed 9483801.

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